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Award Number DAMD17-99-1-9043

TITLE: Regulation of Cell Cyclin Dependent Kinase Inhibitors in the Prostate Cell by RRR-Alpha-Tocopheryl Succinate

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REPORT DATE: July 2000

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2000	Final (1 Feb 99 - 31 Jul 00)

4. TITLE AND SUBTITLE
Regulation of Cell Cyclin Dependent Kinase Inhibitors in the Prostate Cell by RRR-Alpha-Tocopheryl Succinate

5. FUNDING NUMBERS
DAMD17-99-1-9043

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Report contains color photos

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

See page 9

14. SUBJECT TERMS Prostate Cancer

15. NUMBER OF PAGES
18

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction:

Specific Aims

Environmental factors including diet play an important role in the initiation and progression of prostate cancer. Our hypothesis was that vitamin E succinate (VES) inhibits cell cycle progression of prostatic cancer cells and thereby reduces cell proliferation, which may lead to more apoptosis. The aim of the grant was to determine (1) if there is differential regulation of p21 and other cell cyclins in response to VES, and determine if VES will arrest prostate cells at either the G_0-G_1 or G_2-M phase of the cell cycle; (2) if VES exposure is associated with increased apoptosis in prostate cells, or is there just cytostasis alone.

Body:

Background

Studies have indicated that VES decreases cell proliferation(Turely 1997). The direct association between VES and apoptosis would be very important, because it may explain the regulation of proliferation. To better understand the role of VES in prostate cell cycle (especially in cell proliferation and S-phase), direct and indirect effect of VES on cell cycle of prostate cell line PC3 was investigated using flow cytometric analysis and, immunoblot, for cyclin E,cdc2(cyclin dependent cyclin), and p21 expression analysis. VES is a potent inhibitor of neoplastic cells in vitro (Prasad 1992) and in vivo (Kelloff 1994). The exact mechanism of VES in growth inhibition and differentiation is not clear. Studies have shown VES to regulate adenylate cyclase and cAMP-dependent proteins (Sahu 1988) inhibits protein kinase C activity and regulate TGF-B (Tumor Growth Factor) protein production (Turley 1995). Retonoic acid and TGF-B has shown to be a negatively regulate E2F activity during growth inhibition (Schwarz 1995).

Cell cycle progression is largely controlled by pathways that link the cell cycle machinery to the transcription apparatus. In our study we have shown that VES inhibiting the PC3 cell growth at concentration of 10 to 100 ug/ml. However, we used 20ug/ml VES throughout the experiment due to cytotoxic effect at higher dose. Various studies have led to the delineation of a pathway controlling the progression of cells from quiescence, through G1, and into S phase that involves the activation of G1 cyclin-dependent kinases (cdk), inactivation of PCNA (proliferating cell nuclear antigen) and related proteins, and accumulation of p21 transcription factor activity.

Methods

A human prostate adenocarcinoma cell line (PC3) and benign prostate cell line BPH-1 were incubated with VES for 24, 48 and 72 hours. Afterwards, PC3 cells were fixed and stained with propidium iodide for flow cytometry analysis. In parallel experiments, total protein was extracted from PC3 cells and analyzed by western blot for the expression of cyclin E and p21. PC3 a prostate cell line was used to determine the effect of VES on proliferation and gene expression. Before and after VES treatment, PC3 cell lines were evaluated for the expression of the tumor suppressor gene cyclin E and p21. The proliferation of the treated and untreated cells were analyzed. VES was used at concentrations of 10ug/ml to 100 ug/ml for this experiments.

Results

At 24 & 48 hours the number of cells in G0-G1 phase increased by 34% & 39% after

treatment with VES respectively. The number of cells in S phase at 24 and 48 hr decreased by 17% & 35% after treatment with VES respectively. At G2-M phase there were not significant changes comparing the treated sample verses untreated control. Our data suggest that VES exposure directly induces cell arrest in PC3 cells possibly through a G1 cell cycle regulator p21, that might play an important role in apoptosis. Therefore, this might be a common alteration in the cells that undergo apoptosis and cell arrest. The expression of cyclin E and p21 were found to be altered compared to untreated controls. Alteration of cyclin E and p21 and suppression of PCNA was seen in the VES treated samples in a dose dependent manner. PC3 cell death was significantly increased in the treated samples compare to controls. We hypothesize that VES exposure to prostate cells will directly induced cell arrest at G0-G1 checkpoint #1, a critical step for apoptosis. The results described in this study indicate a possible role of cell cycle regulator in the inhibition of proliferation and induction of cell arrest. The role of diet specially the vitamins such as VES may be important in altering the cell cycle pathway and proliferation rate. More study is needed to identify the signal transduction factors responsible for the changes included by VES.

Key Research accomplishments:

-We have established that VES act as a inhibitor of cell cycle
-The effect of VES is at the G1/S phase of the cell cycle
-cyclin E is one of the cell cycle markers that is effected
-Cell death was confirmed with both growth rate and cell death assays
-Obtaining a Benign Prostate cell line to compare the effect of VES in PC3 cells.

Reportable outcomes:

-Abstract has been submitted to AACR-NCI-EORT 1999 meeting in Washington DC.
-Two research assistant and one graduate student were involved in this research and all of them specially the graduate student have trained on cell culture, cell cycle analysis, cyclin E and p21 expression analysis.

Conclusion:

Significance

Our data suggested that VES exposure directly induces cell arrest in PC3 cells possibly through a G1 cell cycle regulator cyclin E and p21, that might play an important role in apoptosis. Therefore, this might be a common alteration in the cells that undergo apoptosis and cell arrest in response to VES. We have recently received a benign prostate cell line BPH-1 from a collaborator at Harvard Medical school. To allow us to compare the effects of VES on a near normal cell. We have little preliminary data on cell cycle changes and proliferation of these cells after treatment with VES. Based on our initial data, there was a differential regulation of cell cycle arrest in PBH-1 compare to PC3 cells. We would like to continue to work on both of these cell lines and a third one, LNcap. In addition to the above aims on this proposal, we would like to investigate the effect of VES on the signal transduction pathways specially ERK1, 2. The results described in this study indicate a possible cell cycle regulator in the inhibition of proliferation and induction of cell arrest. Chemoprevention by natural diets are one of the key element in the

such part of a diet. The role of diet specially the vitamins such as VES may be important in altering the cell cycle pathway and proliferation rate. Additional studies are needed to identify the signal transduction factors responsible for the changes induced by VES. The long range goal of this project will be to analyze the effect of VES on E2F family members which may play distinct roles in cell cycle control, and that E2F1 (Elongation Factor #1) may function as a specific signal for the initiation of apoptosis that would normally be blocked for cell to become tumorigenic.

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Appendices:
Abstract was accepted and published by AACR-NCI-EORT 1999 meeting in Washington DC.

Abstract

Mortality from prostate cancer is the second leading cause of cancer in men. Various studies have led to the delineation of a pathway controlling the progression of cells from quiescence, through G1, and into S phase that involves the activation of G1 cyclin-dependent kinases, and accumulation of transcription factor activity. Diets containing vitamin E are among the protective agent in varies of cancer including prostate cancer and their effects thought to be at the shifting the balance between proliferation and apoptosis. Human prostate adenocarcinoma cell line (PC3) and benign prostate Human (BPH-1) cell lines were used to determine the effect of Vitamin E succinate (VES) on proliferation (by both growth curve and measuring metabolism of soluble tetrazolium compound), by cell death, and by using cell cycle marker. The cells were incubated with and without VES (20ug/ml) for 24, 48 and 72 hours. Afterwards, cells were fixed and stained with propidium iodide for flow cytometry analysis. Cell death was evaluated by trypan blue exclusion dye. In parallel experiments, total protein was extracted from cells and analyzed by western blot for the expression of cyclin E. Overexpression of cyclin E, lower proliferation rate, and cell death were seen in the VES treated samples in a time dependent manner in BPH-1. PC3 and BPH-1cell death were significantly increased at 6% and 26% compare to controls respectively. At 72 hours the number of BPH-1 and PC3 cells in G0-G1 phase increased by 17% & 9% after treatment with VES respectively. The number of BPH-1 cells in S phase at 72 hr decreased by 11% after treatment with VES. At the same time points the changes in cell cycle phases in PC3 were not statistically significant. The number of BPH- 1cell death in the treated samples were increased from two fold to seven fold through 72 hr. Our data suggest that VES exposure directly induces cell arrest in BPH-1 cells possibly through a G1 cell cycle regulator cyclin E, that might play an important role in apoptosis. The effect of VES on PC3 cell cycle, cell proliferation and cell death was less then the benign prostate cell line. This differential regulation might be due to the differentiation level of the two cell lines. The role of diet specially the vitamins such as VES as a chemopreventive agent may be important in altering the cell cycle pathway and proliferation rate. More study is needed to identify the signal transduction factors responsible for the changes included by VES.

Materials and Methods

Cell culture:

PC3 and BPH-1 cell lines were cultured as monolayer inRPMI or M'coys medium supplemented with 10% or 5% fetal bovine serum without antibiotics respectively. Cells were incubated in 5% CO2 at 37o C in humidified air. Cells were exposed to VES suspended in culture medium at a concentration of 20 ug/ml, for 24, 48, and 72 hours. VES was used at 20 ug/ml in 0.1% of ethanol in these experiments.

Cell viability assay:

Cell viability was determined by measuring metabolism of a soluble tetrazolium compound (MTS) and by growth curve. Cell viability was also evaluated by trypan blue exclusion, where dead cells are stained with trypan blue and viable cells remain unstained.

Flow cytometric analysis:

The cells were incubated with and without VES (20ug/ml) for 24, 48 and 72 hours. Afterwards, cells were fixed and stained with propidium iodide for flow cytometry analysis.

Antibody

The cyclinE monoclonal antibody was used at a 1:200 dilution, purchased from Santa Cruze Biot. (Santa Cruze, CA).

Western Blot:

In parallel experiments, total protein was extracted at each time interval, soluble protein was extracted from cells and, analyzed by western blot for the expression of cyclin E.

Results

VES changes the G0-G1 and S phase of the cell cycle consistent with downregulation of proliferation. PC-3 and BPH-1 cells were overlaid with culture medium containing VES for up to 72 h. The cells subjected to flow cytometric analysis. VES exposure to cells increased the percentage of cells in Go-G1 phase by 17% and 9% in BPH-1 and PC-3 respectilvely. The number of the cells in S phase, decreased by 11% in BPH-1 and 8% in PC-3 cells. The decresed in S phase in PC-3 cells were not statistically significant (Figure1).

VES exposure increase cell death:

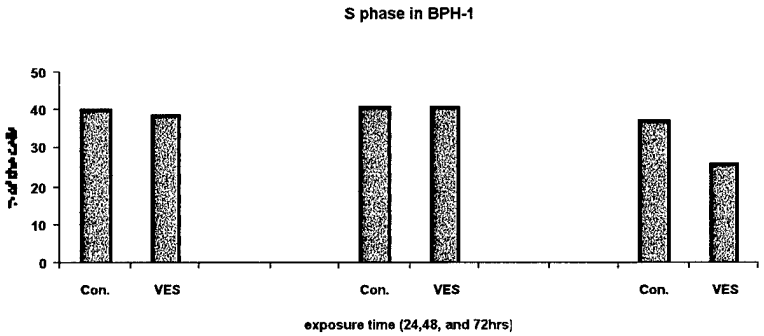
The cell death was incresed by the VES as shown in Figure 2. The number of BPH-1 cell death in the treated samples were increased from two fold to seven fold through 72 hr. The proliferation rate was calculated based on growth rate over 72 hrs. As shown in

VES exposure alter Cyclin E expression:

PC-3 cells were overlaid with culture medium containing20 ug/ml for 24, 48, and 72 h. At each time point, soluble cell protein was extracted from the cells, and cyclin E expression was determined by western blot that demonstrated a gradual increase in cycln E (Figure 4). Densitometer analysis of the westernblot showed a 2.4-fold increase in cyclin E expression over 48h. The increase in cylin expression is consistent with slow down of early S phase in cell cycle in response to VES exposure to PC-3 cells. These data show that VES leads to downregulation of proliferation may progress through a cell cycle pathway.

Discussion

Our data support VES ability to induce cell death, likely through cell cycle growth arrest. The upregulation of cyclin E appears to involve in G1/S phase of cell cycle pathway, leading to suppression of proliferation 72h. of exposure. Here we showed that vit E caused upregulation of cyclin E expression in PC-3 cells by increasing the percentage of cells in G1/S phase, and reducing the percentage of cells in S phase. These observations confirms that more cells were retained in G0-G1 phase of the cell cycle. Cell cycle inhibitors are subject to precise topological control. Therefore, we postulate that Vit E as a cell cycle inhibitor may stimulate the cell to go through growth arrest at G0-G1 phase by upregulation of cyclin E.The effect of VES on PC3 cell cycle, cell proliferation and cell death was less then the benign prostate cell line. This differential regulation might be due to the differentiation level of the two cell lines. The role of diet specially the vitamins such as Vitamin E as a chemopreventive agent may be important in altering the cell cycle pathway. It might play an important role in the balance of proliferation and apoptosis rate. More study is needed to identify the signal transduction factors responsible for the changes included by VES.



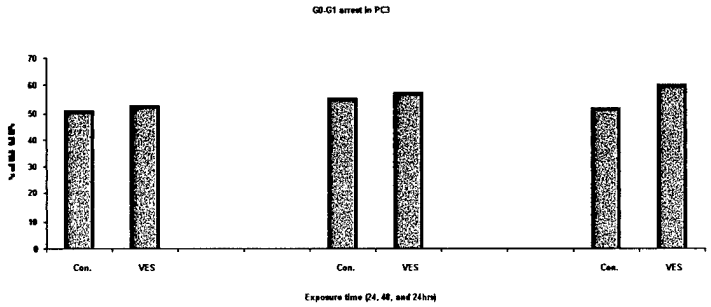
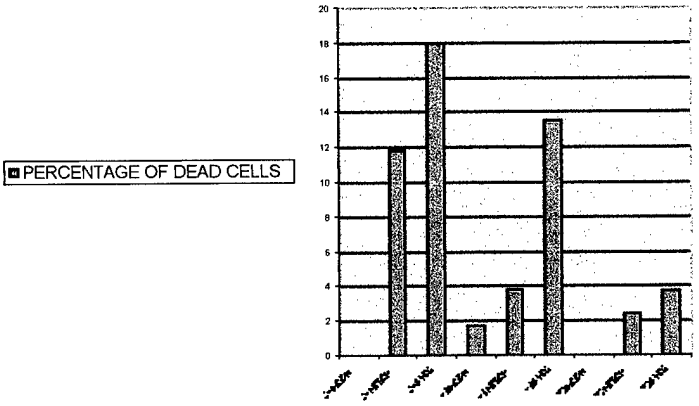


Figure 1. PC-3 and BPH-1 cells cultured were exposed to VES for 24, 48, and 72 h. The cells were fixed in 70% ethanol, and then were stained with PI and subjected to flow cytometric analysis. The data shows the percentage of the cell in G0-G1 and S phase of the cell cycle.

Figure 2

PERCENTAGE OF DEAD CELLS



percentage of dead cells(BPH-1)

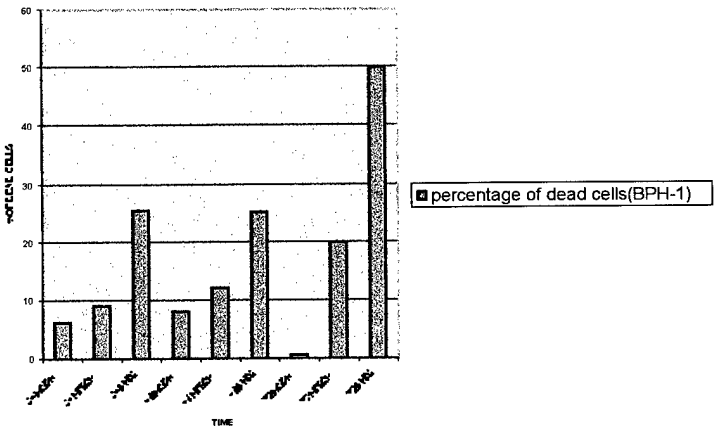
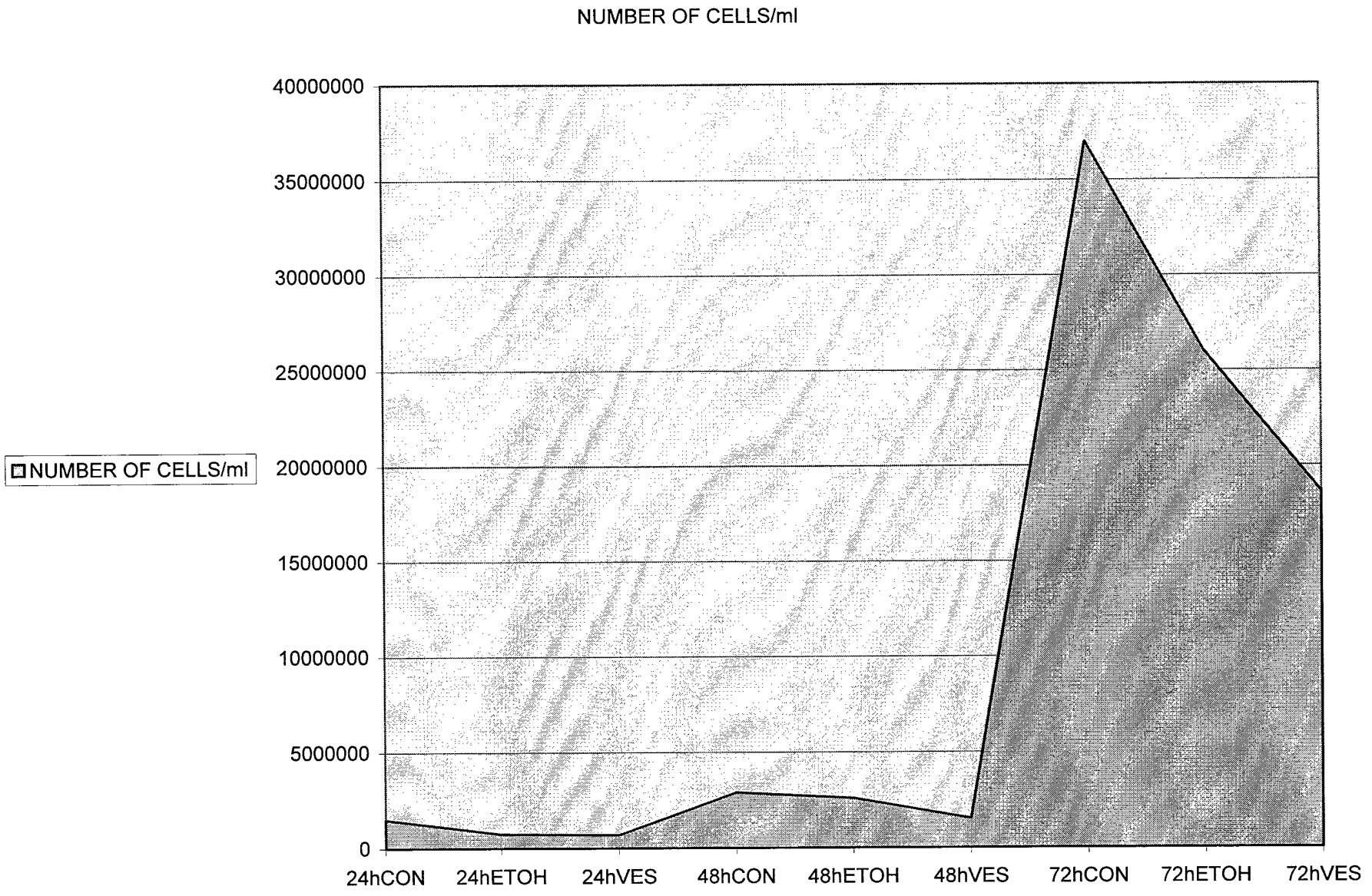


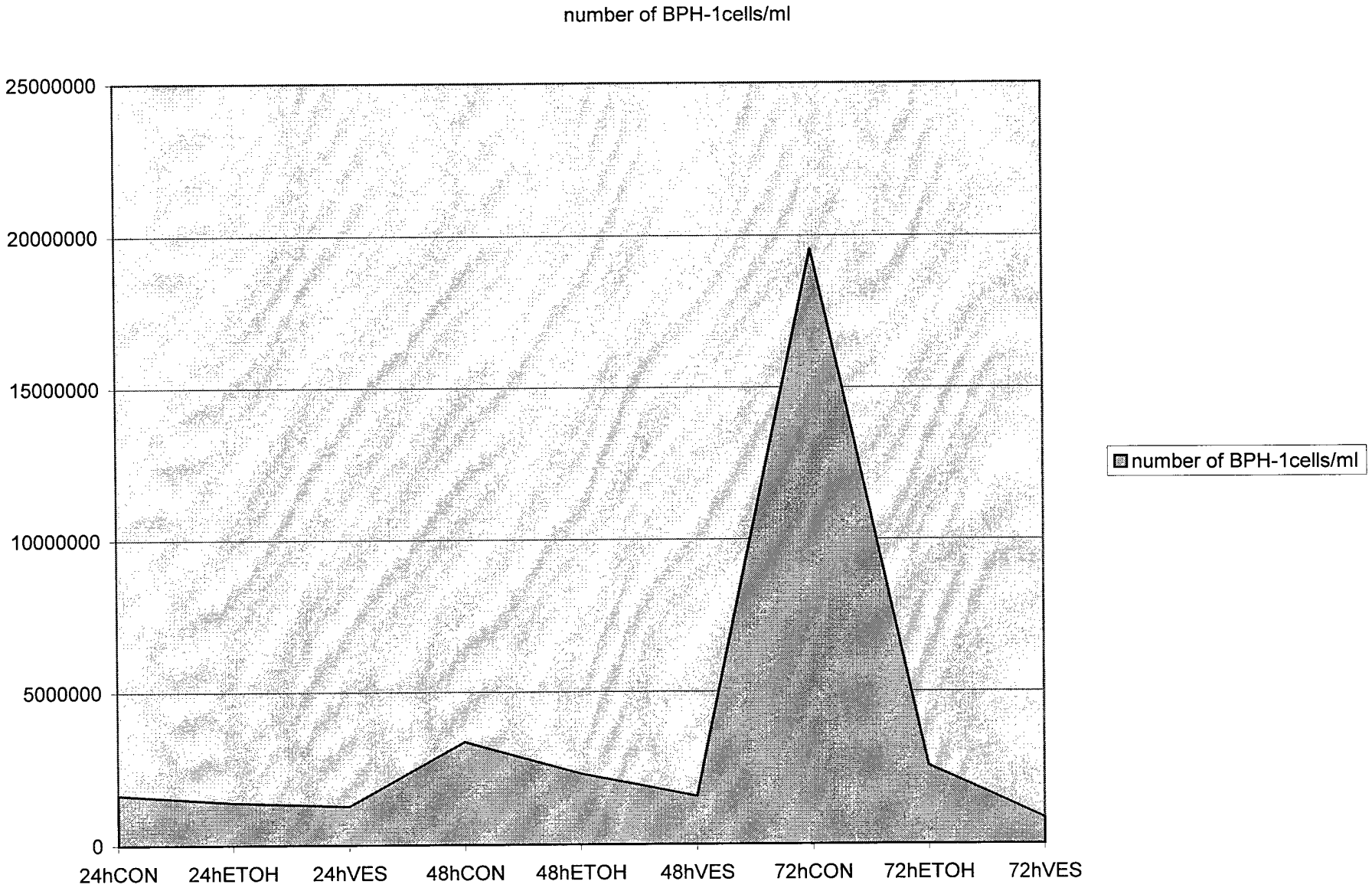
Figure 3a

Figure #3 the growth of Both PC-3 and BPH-1 in presence of VES significantly Suppresed



PC3

Figure 3b


number of BPH-1cells/ml
25000000
20000000
15000000
10000000
5000000
0
24hCON
24hETOH
24hVES
48hCON
48hETOH
48hVES
72hCON
72hETOH
72hVES
number of BPH-1cells/ml

24h 48h

C V C V

Figure 4. Immunobloting analysis of cyclin E in PC-3 cells exposed to vit E for 24, and 48 hours. Total protein was extract and 50 ug of protein was loaded on SDS-PAGE. The protein then were immunoblotted with, anti-human cyclin E C=control, V=VES(50, 42 kDa).

H. Ashktorab, Elghazi, Y., Allen, Neapolitano, M., C., Ahmed, A., Tackey, R., Walters, C.S., and Smoot, D.T. Cancer center, Department of Medicine, Howard University, Washington, D.C.

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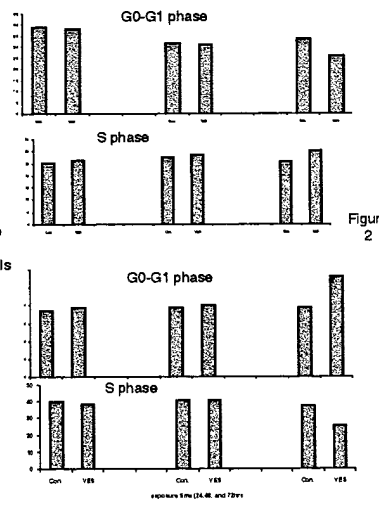

Figure 2

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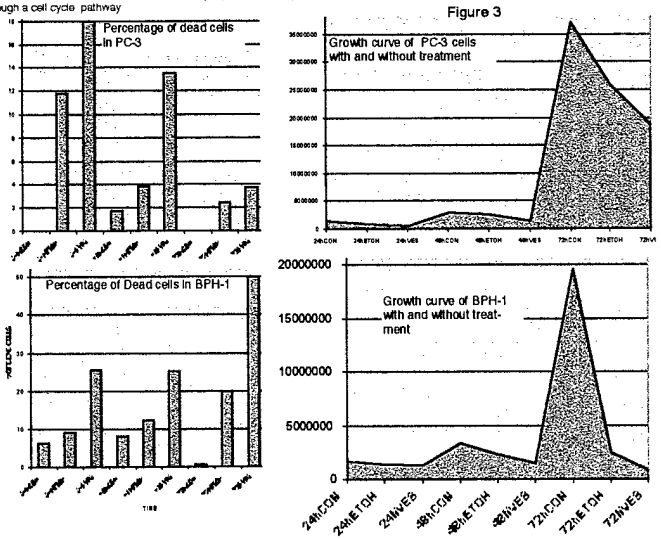

Figure 3

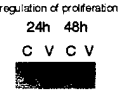

Figure 4. Immunoblotting analysis of cyclin E I 3 cells exposed to vit E for 24, and 48 hours protein was extract and 50 ug of protein was l on SDS-PAGE. The protein then were immur ted with, anti-human cyclin E C=control, V=VI 42 kDa).

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